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Extracellular field recordings

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An abbreviated version of this protocol was published in eLIFE in Oct 2021

NHE6 depletion corrects ApoE4-mediated synaptic impairments and reduces amyloid plaque load

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Detailed protocol

Hippocampal slices were prepared from 3-month-old mice (tamoxifen-injected at 8 weeks). Slices of mice were obtained from four different genotypes; *Slc9a6^{fl};CAG-Cre^{ERT2}* mice or *Slc9a6^{fl}* mice with *Apoe^{APOE3}*or *Apoe^{APOE4}*. Mice were anesthetized with isoflurane and decapitated. The brains were quickly removed and placed in ice-slush high sucrose cutting solution (in mM: 110 sucrose, 60 NaCl, 3 KCl, 1.25 NaH_2PO_4, 28 $NaHCO_3$, 0.5 $CaCl_2$, 5 glucose, 0.6 ascorbic acid, 7 $MgSO_4$), bubbled with a gas mixture of 95% O_2 and 5% CO_2 for oxygenation. 350 µm transverse sections were cut using a vibratome (Leica). Slices were transferred into an incubation chamber containing 50% artificial cerebrospinal fluid (aCSF, in mM: 124 NaCl, 3 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 10 D-glucose, 2 $CaCl_2$, 1 $MgSO_4$) and 50% sucrose cutting solution oxygenated with 95% O_2/5% CO_2 at room temperature for at least 30 minutes. Slices were transferred into an interface chamber continuously oxygenated with 5% Carbon Dioxide USP and 95% Oxygen USP (UN3156) and super fused with aCSF with a speed of 2-3 ml/min in the presence or absence of reelin (2 µg/ml) at 31 °C. Two similar rigs were simultaneously used. Control slices and reelin-treated slices were switched in the second run to avoid timing and chamber differences.

Stimulating electrode was placed on the Schaffer-collateral of the CA1-pyramidal neurons and the recording electrode on the dendrites of the CA3-pyramidal neurons to record field potentials from a population of neurons. For stimulation concentric bipolar electrodes (FHC, catalog no CBBRC75) were placed into the stratum radiatum. An input output(I/O) curve was calculated with increasing stimulus intensities to find the maximum response. Baseline stimulus intensity was set at 40–60% of maximum response and delivered at 33 mHz through an Isolated Pulse Stimulator (A-M Systems, Model 2100). Recording electrodes were prepared using a flaming micropipette puller (Model P-97, Sutter Instruments). The resistance of recording electrodes ranged between 2 and 5 megaohms. Signals were recorded using a microelectrode AC Amplifier (A-M Systems, Model 1800) with a gain of 1000 and filtered between 0.1 and 5KHz. Data was digitized at 10 Khz and transferred via a BNC-2090, National Instruments device to a computer. Once baseline was stably recorded for 20 min, theta burst stimulation (TBS; train of four pulses at 100 Hz repeated 10 times with 200 ms intervals; repeated five times at 10 s intervals, 200 pulses in total in 50 seconds) was applied, and traces were collected with the pre-stimulation intensities for an hour thereafter. Slices with unstable baselines or with more than 10% changes before the TBS protocol were discarded. A custom written program in LabView 7.0 (courtesy of Dr. Jay Gibson, UT Southwestern) was used for recording and analysis of extracellular field experiments.

Data were analyzed offline and fEPSP slopes were calculated as the linear portion of the initial curve. Each experiment was normalized to the average of 20 minutes before TBS. Then those normalized values were used to calculate a mean and standard error. Presynaptic fiber volleys amplitudes are measured and plotted against corresponding fEPSP slopes.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Pohlkamp, T. , Durakoglugil, M. S. and Herz, J. (2021). Extracellular field recordings. Bio-protocol Preprint. bio-protocol.org/prep1440.
2. Pohlkamp, T., Xian, X., Wong, C. H., Durakoglugil, M. S., Werthmann, G. C., Saido, T. C., Evers, B. M., White, C. L., Connor, J., Hammer, R. E. and Herz, J.(2021). NHE6 depletion corrects ApoE4-mediated synaptic impairments and reduces amyloid plaque load. eLIFE. DOI: 10.7554/eLife.72034